Variation of electroencephalographic activity during non-rapid eye movement and rapid eye movement sleep with phase of circadian melatonin rhythm in humans

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1. The circadian pacemaker regulates the timing, structure and consolidation of human sleep. The extent to which this pacemaker affects electroencephalographic (EEG) activity during sleep remains unclear.

2. To investigate this, a total of 1·22 million power spectra were computed from EEGs recorded in seven men (total, 146 sleep episodes; 9 h 20 min each) who participated in a one-month-long protocol in which the sleep–wake cycle was desynchronized from the rhythm of plasma melatonin, which is driven by the circadian pacemaker.

3. In rapid eye movement (REM) sleep a small circadian variation in EEG activity was observed. The nadir of the circadian rhythm of α-activity (8·25–10·5 Hz) coincided with the end of the interval during which plasma melatonin values were high, i.e. close to the crest of the REM sleep rhythm.

4. In non-REM sleep, variation in EEG activity between 0·25 and 11·5 Hz was primarily dependent on prior sleep time and only slightly affected by circadian phase, such that the lowest values coincided with the phase of melatonin secretion.

5. In the frequency range of sleep spindles, high-amplitude circadian rhythms with opposite phase positions relative to the melatonin rhythm were observed. Low-frequency sleep spindle activity (12·25–13·0 Hz) reached its crest and high-frequency sleep spindle activity (14·25–15·5 Hz) reached its nadir when sleep coincided with the phase of melatonin secretion.

6. These data indicate that the circadian pacemaker induces changes in EEG activity during REM and non-REM sleep. The changes in non-REM sleep EEG spectra are dissimilar from the spectral changes induced by sleep deprivation and exhibit a close temporal association with the melatonin rhythm and the endogenous circadian phase of sleep consolidation.

The suprachiasmatic nuclei (SCN) of the hypothalamus are the locus of a circadian pacemaker that plays a pivotal role in the timing of the rest–activity and sleep–wake cycles (Klein, Moore & Reppert, 1991). A primary role of the SCN in sleep–wake regulation in humans is to consolidate wakefulness by generating a signal during the habitual day that counteracts the increase in sleep propensity associated with sustained wakefulness, and to consolidate sleep during the biological night by generating a hypnotic signal that counteracts the increase in sleep satiety associated with sustained sleep (Dijk & Czeisler, 1994, 1995). Electrophysiological, hormonal or biochemical correlates of the endogenous circadian hypnotic signal involved in sleep consolidation have not been identified.

The electroencephalogram (EEG) exhibits marked variations across the vigilance states and serves as a primary variable in sleep research. The recent identification of cellular processes and neural networks underlying the hallmarks of EEG activity during non-rapid eye movement (REM) sleep, i.e. EEG slow waves and sleep spindles, has revitalized interest in the sleep EEG as an indicator of the sleep process and its regulation (Steriade, McCormick & Sejnowski, 1993). Manipulations of sleep propensity by sleep deprivation or hypnotics, as indexed by sleep latency, total sleep time and sleep efficiency, are associated with changes in the sleep EEG. Quantitative analyses of the EEG have revealed that all hypnotics (benzodiazepines and non-benzodiazepines) that bind to the $GABA_A$–benzodiazepine receptor complex

induce similar and characteristic changes in EEG oscillations during non-REM sleep. These changes consist of a reduction of EEG activity at low frequencies, including slow-wave activity (SWA, 0·75–4·5 Hz) and θ-activity, and marked increases in sleep spindle activity (12–15 Hz). Within the frequency range of sleep spindles, lower frequency spindle activity is enhanced the most and high-frequency sleep spindle activity is either reduced or not markedly affected by hypnotics (Borbély, Mattmann, Loepfe, Strauch & Lehmann, 1985; Trachsel, Dijk, Brunner, Klene & Borbély, 1990; Brunner, Dijk, Munch & Borbély, 1991). In contrast, sleep deprivation results in an increase in SWA and θ-activity and a reduction of sleep spindle activity (Dijk, Hayes & Czeisler, 1993). Presently, it is unknown whether sleep occurring during the endogenous circadian phase of high sleep propensity is characterized by changes in the EEG similar to those induced by sleep deprivation or similar to those induced by benzodiazepine hypnotics. A previous qualitative analysis of the circadian modulation of the EEG in non-REM sleep has suggested that activity in the spindle band (12·75–15·0 Hz) varied as a function of circadian phase of the body temperature rhythm, whereas EEG activity in the slow-wave band (0·75–4·5 Hz) was only marginally affected by circadian phase (Dijk & Czeisler, 1995). To quantify the effects of the circadian pacemaker on EEG activity we investigated the circadian modulation of the EEG power spectrum over a broad frequency range (0·25–25·0 Hz) with a 0·5 Hz resolution, separately for non-REM and REM sleep.

The circadian rhythm of plasma melatonin – which in humans is driven by the light-sensitive pacemaker located in the SCN (Shanahan & Czeisler, 1991) – has been implicated in the circadian regulation of sleep (Dollins, Zhadanova, Wurtman, Lynch & Deng, 1994; Tzischinsky & Lavie, 1994). Therefore, we investigated the temporal association between the circadian modulation of EEG oscillations and the melatonin rhythm.

METHODS

Subjects were seven men (age, 21–25 years) who were free of sleep complaints, as assessed by a sleep disorders questionnaire, and in good physical and mental health. All subjects gave their informed consent and the protocol was approved by the Committee for the Protection of Human Subjects from Research Risks at the Brigham and Women's Hospital. Our screening procedures included a physical examination, chest radiography, electrocardiography, biochemical screening and psychological tests such as the Minnesota Multiple Personality Inventory. All subjects were free of drugs, including nicotine, alcohol and caffeine, as assessed by toxicological screening upon arrival in the laboratory. Subjects were instructed to keep a regular sleep–wake schedule for at least 1 week prior to the start of the study. During this period their sleep–wake schedule was verified by ambulatory monitoring of motor activity. For the duration of the study, subjects lived in the laboratory without knowledge of clock time, and rectal temperature was recorded continuously from a disposable thermistor (Yellow Springs Instruments Co., Yellow Springs, OH, USA) and stored at 1 min intervals.

During their approximately 1 month stay in the laboratory, subjects lived on a forced desynchronization protocol. Subjects were scheduled to a 28 h sleep–wake schedule. Sleep was scheduled to occur in darkness for 9·33 h of each 28 h day; during the scheduled waking episodes, light intensity was kept at low levels (10–15 lx). Under these conditions the endogenous circadian pacemaker, which drives the circadian rhythms of body temperature and plasma melatonin, oscillates with a near-stable period that is close to 24 h, and sleep thus occurs at many circadian phases while at the same time variations in wakefulness preceding scheduled sleep episodes are minimized (Czeisler, Allan & Kronauer, 1990; Dijk & Czeisler, 1995).

During selected sections of the protocol, blood samples were drawn at hourly intervals through an indwelling intravenous catheter with side portholes (Deseret Medical Inc., Sandy, UT, USA) placed in a forearm vein. Samples were centrifuged immediately, and the plasma was frozen at −20 °C. Plasma melatonin concentrations were assayed using a radioimmunoassay (Stockgrand Ltd, Surrey, UK). For the present analyses, a total of 1623 plasma samples were assayed. The endogenous circadian phase of the melatonin rhythm was assessed by applying a harmonic regression model to each subject's melatonin data (Brown & Czeisler, 1992).

During all scheduled sleep episodes EEG, EOG (electro-oculogram), EMG and ECG were recorded on Nihon Kohden 5208 or 4418 electroencephalographs. EEGs were derived from electrodes placed on the mastoid and sensory motor areas (C3-A2 and C4-A1). EEG signals were high-pass filtered with a time constant of 0·3 s and low-pass filtered at 35 Hz. The EEG signals derived from C3-A2 and C4-A1 were digitized at a sampling rate of 128 Hz using a 12 bit external A/D convertor (Digital Laboratory Peripheral Accelerator; LPA-11) and stored on a VAX 11/750 computer (Digital Equipment Corp., Maynard, MA, USA). EEGs were subjected to a spectral analysis by a IMSL Fast Fourier Transform routine in which a Parzen spectral window was implemented (IMSL Library, *Fortran Subroutines for Mathematics and Statistics* (1984), IMSL Inc., Houston, Texas, USA). Power spectra were calculated for 4 s epochs, which resulted in a resolution of 0·25 Hz. A total of more than 1·22 million spectra were computed. Data were reduced by collapsing 0·25 Hz bins into 0·5 Hz bins, omitting spectra above 25 Hz, and averaging over alternately seven or eight 4 s epochs, resulting in one power spectrum every 28 or 32 s. Power spectra were calibrated by recording a 16 Hz, 25 μV sine wave calibration signal prior to each sleep episode. All polysomnograms were scored over 30 s epochs according to standard criteria. EEG power spectra were synchronized with the sleep stages, and epochs contaminated by artifacts were visually identified and excluded from the analysis. Power spectra and sleep stages were transferred to the hard disk of a PC. Sleep stage-specific spectra of one derivation (usually C3-A2) and their circadian and sleep-dependent modulation were calculated with software written in PASCAL. For the computation of sleep stage-specific spectra, average power spectra were calculated for non-REM sleep, REM sleep and total sleep (stage 1 included) over all sleep episodes in each subject separately. All power density values were expressed as a percentage of total power (0·25–30·0 Hz) in total sleep in each subject separately, and then averaged across subjects (Fig. 1). All initial analyses were performed on 0·5 Hz bands rather than the commonly used broader frequency bands such as δ, θ, σ etc. in order to establish that no discontinuities related to circadian- and sleep-dependent influences on EEG activity within these bands existed. For visual representation of the circadian- and sleep-dependent variations, data were presented either: (1) as activity within commonly used broad bands when no discontinuities were detected; or (2) in

narrower frequency bands when a discontinuity was detected within a band, as was the case within the σ-band.

The SAS® software package version 6.03 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. Data were analysed with one- or two-factor ANOVAs, with factors *circadian phase* and *time elapsed since start of sleep episode*. When possible, repeated measures ANOVAs were applied.

The strength of the circadian- and sleep-dependent influence on power density values in non-REM sleep and REM sleep was computed according to the following method. For each 30 s epoch of sleep, *circadian phase* and *time elapsed since start of sleep episode* were computed. In each subject the average power spectrum was computed for non-REM and REM sleep separately, and for each 30 s epoch, power in each frequency bin was expressed as a deviation from the mean power in this frequency bin and sleep stage. Next, for each subject, sleep stage (i.e. non-REM or REM sleep) and frequency bin, power density values were sorted in a two-dimensional 8 × 4 matrix representing eight *circadian phase* bins of 45 deg each, and four *time elapsed since start of sleep episode* bins of 140 min each. For each subject an average value was computed for each of the thirty-two cells. This procedure removes variation associated with the non-REM–REM cycle and possible ultradian variations superimposed on the circadian modulation. Next, the average values were entered in a two-factor ANOVA with factors *circadian phase* (eight 45 deg bins) and *time elapsed since start of sleep episode* (four 140 min intervals). F values and the percentage of the variance explained by these factors (i.e. 100 × the factor sum of squares/total sum of squares) were then computed.

To illustrate the circadian- and sleep-dependent variations in more detail, these data were plotted with a higher resolution (a 2 h and a 112 min resolution for the circadian- and sleep-dependent time courses, respectively). With this higher resolution, data could not be subjected to a two-way ANOVA because not all of the subjects contributed to the sixty (i.e. 12 × 5) cells. However, one-factor ANOVAs on these data yielded results that were virtually identical to the two-way ANOVA. Here we only report the results from the two-way ANOVA.

RESULTS

In all subjects, the circadian rhythm of plasma melatonin oscillated with a period that was close to 24 h and similar to the period of the circadian rhythm of body temperature. Power spectra in non-REM and REM sleep exhibited the typical sleep stage-specific characteristics, with a predominance of EEG activity at low frequencies and in the frequency range of sleep spindles (12–15 Hz) during non-REM sleep (Fig. 1*A*), and lower values in these same frequency ranges during REM sleep (Fig. 1*D*). The relative contribution of circadian phase and sleep-dependent processes to the EEG varied widely across the analysed frequency range (0·25–25·0 Hz) and between non-REM and REM sleep.

In REM sleep, the circadian variation of EEG activity was statistically significant in the 0·75–4·0, 6·75–10·5 and 12·75–14·0 Hz frequency ranges ($P < 0·01$ in all cases). It was most pronounced in the low α-frequency range (8·25–10·5 Hz), and the trough of this circadian rhythm was located close to the end of the interval during which plasma melatonin values were high, i.e. at the crest of the REM sleep propensity rhythm (Fig. 2). In the course of the sleep episode, EEG activity in REM sleep decreased significantly in the frequency ranges of 1·25–8·0 and 10·75–19·5 Hz.

In non-REM sleep, the circadian variation of EEG activity was statistically significant ($F_{7,192}$; $P < 0·01$) in the 1·25–10·5 Hz and 11·25–16·5 Hz ranges (Fig. 1*C*). The low-amplitude circadian rhythms in the slow-wave, θ- and α-frequency bands exhibited crests at approximately 210–240 deg, i.e. before the rise of plasma melatonin concentrations, and before the phase of high sleep consolidation (Figs 2 and 3).

In the frequency range of sleep spindles, the circadian variation was high in both the 12·25–13·0 and 14·25–15·5 Hz ranges and lower in the 13·25–14·0 Hz range (Fig. 1*C*). The crest of the high-amplitude circadian rhythm of low-frequency sleep spindle activity was located close to the crest of the educed circadian rhythm of plasma melatonin, whereas the crest of the circadian rhythm of high-frequency sleep spindle activity coincided with the nadir of the circadian rhythm of plasma melatonin. These changes in EEG power density in non-REM sleep were not directly associated with the circadian variation in the duration of non-REM sleep (Figs 2 and 3).

In non-REM sleep the effect of the factor *time elapsed since start of sleep episode* was significant ($F_{3,192}$; $P < 0·01$) between 0·25–11·5 and 12·75–15·0 Hz. The variation explained by the factor *time elapsed since start of sleep episode* reached high values especially in the slow-wave and θ-bands (Fig. 1*B*), and slow-wave, θ- and α-activity all decreased in the course of sleep (Fig. 3). In contrast, low-frequency, intermediate-frequency (13·25–13·5) and high-frequency sleep spindle activity all increased with the progression of sleep (Fig. 3).

A significant interaction ($F_{21,192}$; $P < 0·01$) between the factors *time elapsed since start of sleep episode* and *circadian phase* was observed only for EEG activity at 12·75–13·0 Hz in non-REM sleep.

The changes in the power spectra of the EEG during non-REM sleep that were associated with the presence of high melatonin concentrations were further investigated by a direct comparison of EEGs recorded when sleep occurred within the circadian phase of melatonin secretion, with EEGs recorded when sleep occurred outside the phase of melatonin secretion. During the melatonin secretory phase, i.e. the circadian phase during which circadian sleep propensity was high, EEG power spectra were characterized by slightly but significantly lower values within the frequency range of 1·25–10·0 Hz. During this phase, low-frequency sleep spindle activity was, on average, 150% of the value observed during sleep when circulating melatonin was at very low levels, whereas high-frequency sleep spindle activity was, on average, only 70% (Fig. 4).

DISCUSSION

The data show that during forced desynchronization of the rest–activity cycle and endogenous circadian rhythms, sleep consolidation remains associated with the rhythm of plasma melatonin, and the endogenous circadian pacemaker has a strong influence upon specific electroencephalographic oscillations during non-REM sleep and a weaker influence during REM sleep. These circadian changes in EEG power spectra are not directly associated with the circadian variation in the duration of these sleep stages. When sleep coincides with the circadian phase of melatonin secretion and sleep is highly consolidated, the EEG in non-REM sleep is characterized by very moderate reductions in the frequency range of slow waves and θ-activity, and profound changes in the frequency range of sleep spindles. These data confirm and extend earlier assessments of the relative contribution of circadian- and sleep-dependent processes to electroencephalographic oscillations (Dijk & Czeisler, 1995). In particular these findings re-emphasize that low-frequency oscillations in non-REM sleep are primarily dependent on the progression of the sleep process (Dijk, Brunner & Borbély, 1990*b*). In addition these findings confirm that in non-REM sleep EEG oscillations between 0·75 and 11·0 Hz all change in a similar way (Dijk, Brunner, Beersma & Borbély, 1990*a*). Furthermore, these data show that the influence of the endogenous circadian pacemaker on EEG activity within the frequency range of sleep spindles is specific for non-REM sleep, since in REM sleep variations of a similar magnitude were not observed.

The observation that the circadian rhythms of low- and high-frequency sleep spindle activity are approximately 180 deg out of phase is in accordance with the recently reported time course of low- and high-frequency sleep spindle activity during sleep displacement experiments

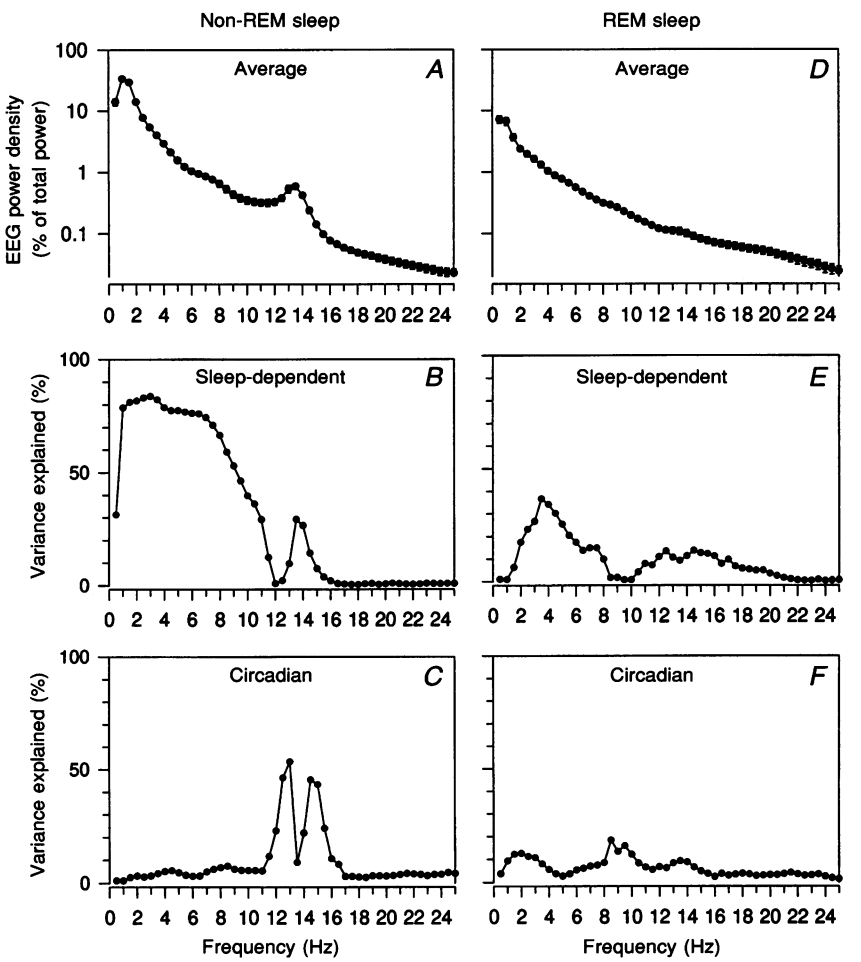

Figure 1. Effect of *circadian phase* and *time elapsed since start of sleep episode* on EEG power spectra in non-REM sleep (left-hand panels) and REM sleep (right-hand panels)

A and *D*, average EEG power spectra (see Methods for detailed description). Values are plotted at the upper limit of the 0·5 Hz bins. Data above 25 Hz are not shown. Note the logarithmic scale on the ordinate. S.E.M. values are indicated but are in general smaller than the size of the symbols. *B* and *E*, percentage of variance of power spectra in non-REM and REM sleep explained by the factor *time elapsed since start of sleep episode*. *C* and *F*, percentage of variance of power spectra in non-REM and REM sleep explained by the factor *circadian phase*.

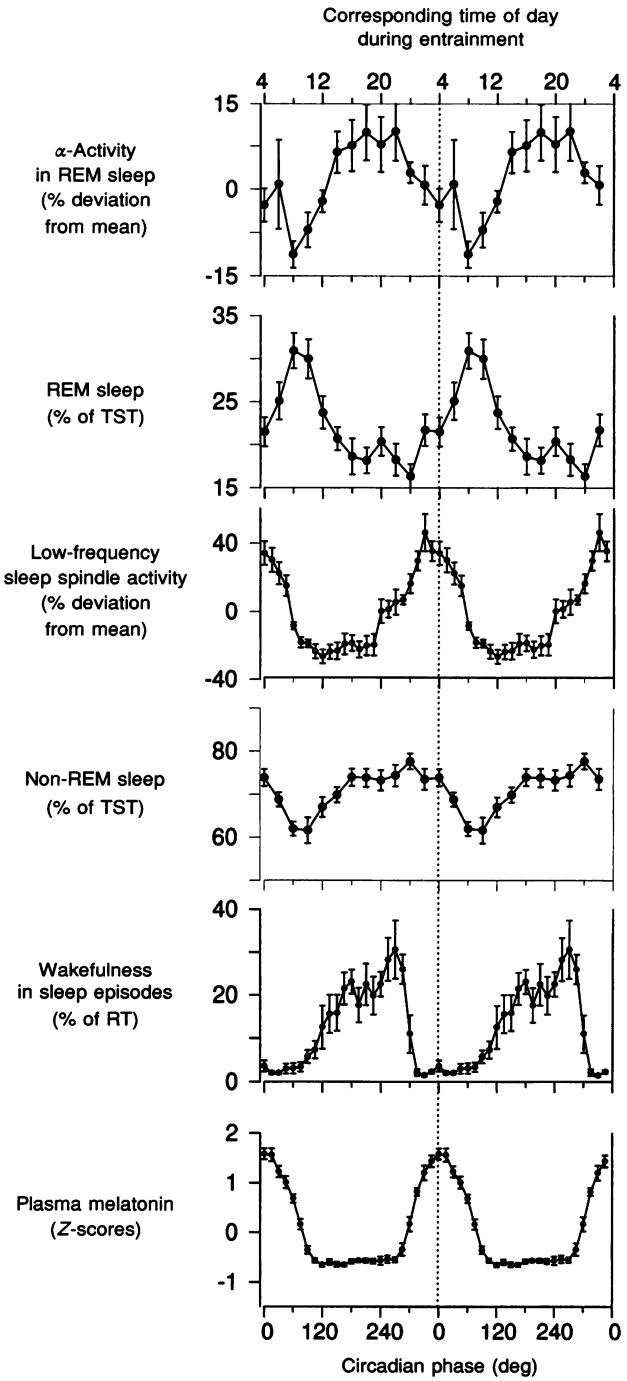

Figure 2. Phase relationships between the circadian rhythms of plasma melatonin, sleep consolidation, non-REM sleep, REM sleep and EEG activity

Data are plotted against circadian phase of the plasma melatonin rhythm (0 deg corresponds to the fitted maximum, bottom x-axis). To facilitate comparison with the situation in which the circadian system is entrained to the 24 h day, the top x-axis indicates the average clock time of the circadian melatonin rhythm during the first day of the forced desynchronization protocol, i.e. immediately upon release from entrainment. Plasma melatonin data were expressed as Z-scores to correct for interindividual differences in mean values. Wakefulness is expressed as a percentage of recording time (RT). Non-REM sleep and REM sleep are expressed as a percentage of total sleep time (TST). Low-frequency sleep spindle activity in non-REM sleep and α-activity in REM sleep are expressed as percentage deviation from the mean. Data are double plotted, i.e. all data plotted left from the dashed vertical line are repeated to the right of this vertical line.

(Aeschbach, Dijk & Borbély, 1997). Such findings may be interpreted in two ways: (1) as a modulation of the frequency of sleep spindles; or (2) as a modulation of two types of sleep spindles with different frequencies. It has been reported that low-frequency spindle activity is most prominent in frontal brain regions whereas high-frequency spindle activity is dominant in EEG recordings from parietal and occipital areas. Both types of spindle activity are present in the central areas from which the present EEG recordings were obtained (Jankel & Niedermeyer, 1985; Scheuler, Kubicki, Scholz & Marquardt, 1990; Werth, Achermann, Dijk & Borbély, 1997). EEG power density in

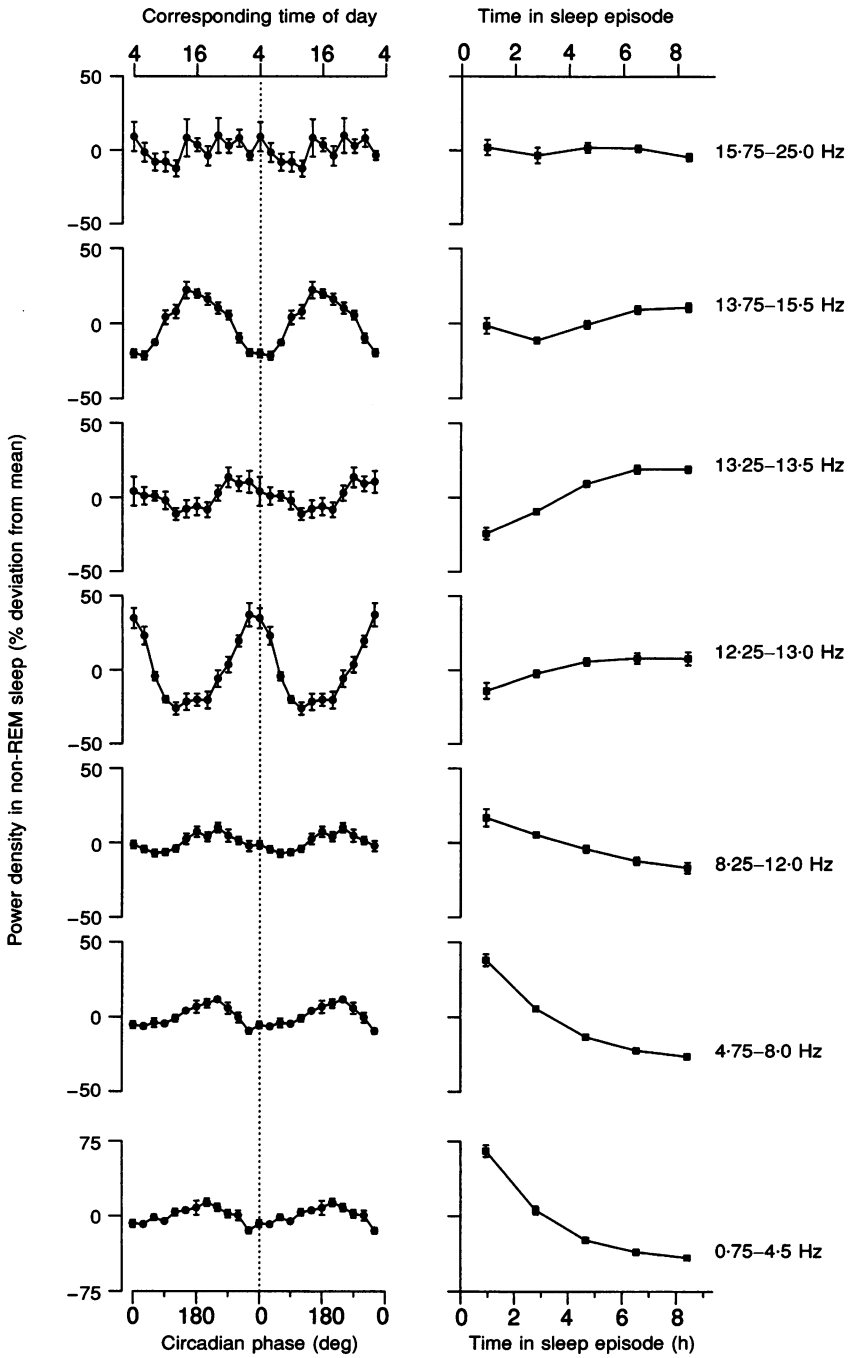

Figure 3. Circadian- and sleep-dependent variation in power density in non-REM sleep

For each band and each subject the circadian- and sleep-dependent effects were first calculated per 0·5 Hz and expressed as percentage deviation from the mean. Next, these relative values were averaged over bins to form the bands shown, and then averaged over subjects. Data are plotted at the mid-points of the circadian bins (30 deg) and time intervals (112 min). Data in the left-hand panels are double plotted. The fitted maximum of the melatonin rhythm was assigned a reference value of 0 deg. Full scale of ordinates is −50 to 50 % for all panels except for the panels in which the 0·75–4·5 Hz band is displayed.

the 12–15 Hz range has been previously shown to be closely correlated with spindle frequency activity as assessed by transient pattern recognition software (Dijk, Hayes & Czeisler, 1993). However, spectral analysis does not allow assessment of the frequency of individual spindles. Therefore we cannot exclude the possibility that the present changes in power density in the spindle frequency range may represent a circadian modulation of the frequency of one type of sleep spindle. Such a modulation of the frequency of sleep spindles may be mediated by a modulation of the duration of the IPSP-rebound sequences in thalamic nuclei (Steriade, Domich & Oakson, 1986).

The circadian variation in the EEG in REM sleep was modest, and most pronounced in the α-frequencies. The circadian waveform of α-activity was quite different from the rhythm of wakefulness within scheduled sleep episodes, which makes it unlikely that this circadian rhythm is primarily related to 'intermittent wakefulness' within 30 s REM sleep epochs. A contribution of very short arousals within REM sleep, scored according to established citeria, cannot, however, be excluded.

The observed pattern of change in the EEG power spectra when sleep coincided with the phase of melatonin secretion and with the phase of high sleep consolidation, i.e. reduction of slow waves and enhancement of low-frequency sleep spindle activity, is different from the change induced by an increase in sleep propensity associated with a prolongation of wakefulness, i.e. sleep deprivation. After sleep deprivation, low-frequency sleep spindle activity (recorded from central derivations referenced against the mastoid) is not markedly affected, intermediate- and high-frequency sleep spindle activity are reduced and slow-wave and θ-activity are enhanced (Dijk *et al.* 1993).

The changes in the EEG associated with the circadian rhythm of plasma melatonin are similar to the changes in EEG power spectra induced by benzodiazepine, imadazopyridine and cyclopyrrolone hypnotics, which all bind to the benzodiazepine–$GABA_A$ receptor complex (Borbély *et al.* 1985; Trachsel *et al.* 1990; Brunner *et al.* 1991). Melatonin's effects are believed to be mediated by G-protein-coupled melatonin receptors (Reppert, Weaver & Ebisawa, 1994) but data in rats indicate that melatonin also potentiates the effects of GABA selectively at $GABA_A$ receptors with a benzodiazepine-like non-competitive agonistic action on thalamic neurons (Tenn & Niles, 1995).

The present data demonstrate only a temporal association between the circadian rhythms in EEG activity in non-REM sleep, sleep consolidation and the rhythm of plasma melatonin. There is increasing evidence for a causal role of melatonin in the generation of these rhythms. Melatonin, when administered outside the phase of endogenous secretion, has been reported to increase sleep propensity (Dollins *et al.* 1994; Tzischinsky & Lavie, 1994); induce changes in the EEG power spectrum during sleep that are to some extent similar to those observed during the biological night, i.e. suppression of slow-wave activity and enhancement of sleep spindle activity (Dijk *et al.* 1995); suppress visually scored slow-wave sleep (Hughes & Badia, 1997) and induce changes in the EEG during wakefulness (Cajochen, Kräuchi, von Arx, Möri, Graw & Wirz-Justice, 1996).

The functional significance of sleep spindles has been debated, but they can be considered to be major inhibitory events that effectively block the transfer of sensory information through thalamic relay nuclei to cortical areas (Jankel & Niedermeyer, 1985; Steriade *et al.* 1993). Circadian modulation of sleep spindles may thus represent a mechanism by which the circadian pacemaker modulates sensitivity to sensory information and hence arousability during sleep.

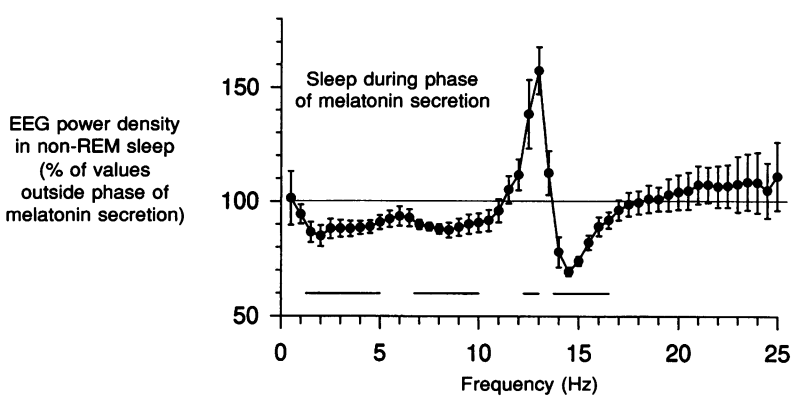

Figure 4. Power spectra in non-REM sleep during sleep occurring between −90 and 90 deg of the endogenous circadian rhythm of plasma melatonin

Under entrained conditions sleep occurring between −90 and 90 deg of the endogenous circadian rhythm of plasma melatonin corresponds to approximately 22:00–10:00 h. Values are expressed relative to 'day sleep', i.e. sleep occurring between 90 and 270 (i.e. −90) deg. 0 deg is the phase of melatonin maximum. Vertical bars represent S.E.M. values. Horizontal lines above abscissa indicate significant differences between night and day values ($P < 0{\cdot}05$; Student's paired t test on log transformed values).

In conclusion, the present data demonstrate that the EEG during non-REM and REM sleep, as well as during sleep consolidation, exhibits significant variations with the endogenous circadian phase of the plasma melatonin rhythm which, in turn, is driven by the suprachiasmatic nucleus.

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